SUMMARY OF THE INVENTION

The present invention provides an immunogenic target for administration to a patient to prevent and / or treat cancer. In particular, the immunogenic target is a tumor antigen ("TA") and / or an angiogenesis-associated antigen ("AA"). In one embodiment, the immunogenic target is encoded by **SEQ ID NO.: 5** or has the amino acid sequence of **SEQ ID NO.: 6**. In certain embodiments, the TA and / or AA are administered to a patient as a nucleic acid contained within a plasmid or other delivery vector, such as a recombinant virus. The TA and / or AA may also be administered in combination with additional tumor antigens (i.e., SEQ ID NOS.: 1-4) and / or an immune stimulator, such as a co-stimulatory molecule or adjuvant.

BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1. BFA4 cDNA sequence (SEQ ID NO.:1).

Figure 2. BFA4 amino acid sequence (SEQ ID NO.:2).

Figure 3. BCY1 nucleotide (A; SEQ ID NO.:3) and amino acid (B; SEQ ID NO.:4) sequences.

Figure 4. BFA5 cDNA sequence (SEQ ID NO.:5).

Figure 5. BFA5 amino acid sequence (SEQ ID NO.:6).

DETAILED DESCRIPTION

The present invention provides reagents and methodologies useful for treating and / or preventing cancer. All references cited within this application are incorporated by reference.

In one embodiment, the present invention relates to the induction or enhancement of an immune response against one or more tumor antigens ("TA") to prevent and / or treat cancer. In certain embodiments, one or more TAs may be combined. In preferred embodiments, the immune response results from expression of a TA in a host cell following administration of a nucleic acid vector encoding the tumor antigen or the tumor antigen itself in the form of a peptide or polypeptide, for example.

As used herein, an "antigen" is a molecule such as a polypeptide or a portion thereof that produces an immune response in a host to whom the antigen has been administered. The immune response may include the production of antibodies that bind to at least one epitope of the antigen and / or the generation of a cellular immune response against cells expressing an epitope of the antigen. The response may be an enhancement of a current immune response by, for example, causing increased antibody production, production of antibodies with increased affinity for the antigen, or an increased or more effective cellular response (i.e., increased T cells or T cells with

metal binding domains (e.g., a poly-histidine segment), immunoglobulin binding domains (i.e., Protein A, Protein G, T cell, B cell, Fc receptor, or complement protein antibody-binding domains), sugar binding domains (e.g., a maltose binding domain), and/or a "tag" domain (i.e., at least a portion of α-galactosidase, a strep tag peptide, a T7 tag peptide, a FLAG peptide, or other domains that can be purified using compounds that bind to the domain, such as monoclonal antibodies). This tag is typically fused to the polypeptide upon expression of the polypeptide, and can serve as a means for affinity purification of the sequence of interest polypeptide from the host cell. Affinity purification can be accomplished, for example, by column chromatography using antibodies against the tag as an affinity matrix. Optionally, the tag can subsequently be removed from the purified sequence of interest polypeptide by various means such as using certain peptidases for cleavage. As described below, fusions may also be made between a TA and a co-stimulatory components such as the chemokines CXC10 (IP-10), CCL7 (MCP-3), or CCL5 (RANTES), for example.

A fusion motif may enhance transport of an immunogenic target to an MHC processing compartment, such as the endoplasmic reticulum. These sequences, referred to as tranduction or transcytosis sequences, include sequences derived from HIV tat (see Kim et al. 1997 J. Immunol. 159:1666), *Drosophila* antennapedia (see Schutze-Redelmeier et al. 1996 J. Immunol. 157:650), or human period-1 protein (hPER1; in particular, SRRHHCRSKAKRSRHH (SEQ ID NO: 105).

In addition, the polypeptide or variant thereof may be fused to a homologous polypeptide to form a homodimer or to a heterologous polypeptide to form a heterodimer. Heterologous peptides and polypeptides include, but are not limited to: an epitope to allow for the detection and/or isolation of a fusion polypeptide; a transmembrane receptor protein or a portion thereof, such as an extracellular domain or a transmembrane and intracellular domain; a ligand or a portion thereof which binds to a transmembrane receptor protein; an enzyme or portion thereof which is catalytically active; a polypeptide or peptide which promotes oligomerization, such as a leucine zipper domain; a polypeptide or peptide which increases stability, such as an immunoglobulin constant region; and a polypeptide which has a therapeutic activity different from the polypeptide or variant thereof.

In certain embodiments, it may be advantageous to combine a nucleic acid sequence encoding an immunogenic target, polypeptide, or derivative thereof with one or more nucleic acid sequences encoding one or more co-stimulatory component(s) such as cell surface proteins, cytokines or chemokines in a composition of the present invention. The co-stimulatory component may be included in the composition as a polypeptide or as a nucleic acid encoding the

TABLE III

BFA5 Peptide Pools

Peptide Group	CLP number	Sequence	SEQ ID	Peptide Group	CLP number	Sequence	SEQ ID
BFA5 Group 1	2983	LMDMQTFKA	7	BFA5 Group 6	3033	FESSAKIQV	53
	2984	KVSIPTKAL	8		3034	GVTAEHYAV	54
	2985	SIPTKALEL	9		3035	RVTSNKTKV	55
	2986	LELKNEQTL	10		3036	TVSQKDVCV	56
	2987	TVSQKDVCL	11		3037	KSQEPAFHI	57
	2988	SVPNKALEL	12		3038	KVLIAENTM	58
	2989	CETVSQKDV	13		3039	MLKLEIATL	59
	2990	KINGKLEES	14		3040	EILSVVAKL	60
	2991	SLVEKTPDE	15		3041	MLKKEIAML	61
	2992	SLCETVSQK	16		3042	LLKEKNEEI	62
BFA5 Group 2	2993	EIDKINGKL	17	BFA5 Group 7	3043	ALRIQDIEL	63
	2994	MLLQQNVDV	18		3044	KIREELGRI	64
	2995	NMWLQQQLV	19		3045	TLKLKEESL	65
	2996	FLVDRKCQL	20		3046	ILNEKIREE	66
	2997	YLLHENCML	21		3047	VLKKKLSEA	67
	2998	SLFESSAKI	22		3048	GTSDKIQCL	68
	2999	KITIDIHFL	23		3049	GADINLVDV	69
	3000	QLQSKNMWL	24		3050	ELCSVRLTL	70
	3001	SLDQKLFQL	25		3051	SVESNLNQV	71
	3002	FLLIKNANA	26		3052	SLKINLNYA	72

Peptide Group	CLP number	Sequence	SEQ ID	Peptide Group	CLP number	Sequence	SEQ ID
BFA5 Group 3	3003	KILDTVHSC	27	BFA5 Group 8	3053	KTPDEAASL	73
	3004	SLSKILDTV	28		3054	ATCGMKVSI	74
	3005	ILIDSGADI	29		3055	LSHGAVIEV	75
	3006	KVMEINREV	30		3056	EIAMLKLEI	76
	3007	KLLSHGAVI	31		3057	AELQMTLKL	77
	3009	AVYSEILSV	32		3058	VFAADICGV	78
	3010	KMNVDVSST	33		3060	PAIEMQNSV	79
	3011	ILSVVAKLL	34		3061	EIFNYNNHL	80
	3012	VLIAENTML	35		3062	ILKEKNAEL	81
BFA5 Group 4	3013	KLSKNHQNT	36	BFA5 Group 9	3063	QLVHAHKKA	82
	3014	SLTPLLLSI	37		3065	NIQDAQKRT	83
	3015	SQYSGQLKV	38		3066	NLVDVYGNM	84
	3016	KELEVKQQL	39		3067	KCTALMLAV	85
	3017	QIMEYIRKL	40		3068	KIQCLEKAT	86
	3018	AMLKLEIAT	41		3069	KIAWEKKET	87
	3019	VLHQPLSEA	42		3070	IAWEKKEDT	88
	3020	GLLKATCGM	43		3071	VGMLLQQNV	89
	3021	GLLKANCGM	44		3072	VKTGCVARV	90
	3022	QQLEQALRI	45	BFA5 Group 10	3074	ALHYAVYSE	91
BFA5 Group 5	3023	CMLKKEIAM	46				
	3024	EQMKKKFCV	47		3075	QMKKKFCVL	92
	3025	IQDIELKSV	48		3076	ALQCHQEAC	93
	3026	SVPNKAFEL	49		3077	SEQIVEFLL	94
	3027	SIYQKVMEI	50		3078	AVIEVHNKA	95
	3028	NLNYAGDAL	51		3079	AVTCGFHHI	96
	3029	AVQDHDQIV	52		3080	ACLQRKMNV	97
					3081	SLVEGTSDK	98

ELISPOT analysis was performed on human T-cell cultures activated through four rounds of stimulation with each pool of BFA5 peptides. Reactivity against a CMV pp65 peptide and a Flu matrix peptide were used as positive controls for T-cell activation in the experiments. Each experiment was performed with PBMC and dendritic cells from a single HLA-A*0201^{+} donor designated as "AP10". The results show that, although BFA4 is markedly reactive with high ELISPOT counts per 100,000 cells in the assay, BFA5 is even more reactive with 9/10 pools demonstrating ELISPOT reactivity. Similar results were obtained for both BFA4 and BFA5/NYBR-1 with a different HLA-A*0201. The bars reach a maximum at 600 spots because beyond that the ELISPOT reader does not give accurate counts. Cultures having a reading of 600 spots have more than this number of spots.

A large number of the BFA5 peptide pools of are reactive as shown by the high levels of IFN-γ production. Each reactive peptide pool was then separated into individual peptides and analyzed for immunogencity using ELISPOT analysis to isolate single reactive BFA5 peptides. BFA5 is highly immunogenic with several reactive single peptides than that of BFA4. Similar results were obtained in two independent PBMC culture experiments.

In addition to ELISPOT analysis, human T cells activated by BFA5 peptides were assayed to determine their ability to function as CTL. The cells were activated using peptide-pulsed dendritic cells followed by CD40 ligand-activated B cells (5 rounds of stimulation). The experiment shown was performed with isolated PBMC from HLA-A*0201^{+} donor AP31. Isolated T cells were tested in ^{51}Cr-release assays using peptide-loaded T2 cells. The % specific lysis at a 10:1, 5:1, and 1:1 T-cell to target ratio is shown for T2 cells pulsed with either pools of BFA5/NYBR-1 peptides or with individual peptides. The graph shows CTL activity induced against targets loaded with a c non-specific HLA-A*0201-binding HIV peptide (control) followed by the CTL activity against the peptide pool (Pool 1 etc.) and then the activity induced by individual peptides from the respective pool to the right. A high level of cytotoxicity was observed for some peptides at a 1:1 E:T ratio. CTL activity (percent specific lysis) induced by the control HIV peptide was generally <10%. Similar results were obtained with another PBMC donor expressing HLA-A*0201 (AP10). A large number of BFA5 peptides trigger T cell-mediated cytotoxicity of BFA5 peptide-loaded target cells. **Table IV** lists those peptides having immunogenic properties. Five peptides (LMDMQTFKA (SEQ ID NO.:7), ILIDSGADI (SEQ ID NO.:29), ILSVVAKLL (SEQ ID NO.:34), SQYSGQLKV (SEQ ID NO.:38), and ELCSVRLTL (SEQ ID NO.:70)) were found to induce both IFN-γ secretion and CTL activity in T cells from both donors.

TABLE IV

Immunoreactive peptides from BFA5

	BFA5 peptides eliciting high IFN-γ release (>200 spots / 100,000 cells)		BFA5 peptides inducing CTL lysis of pulsed cells	
SEQ ID NO.	Donor AP10	Donor AP31	Donor AP10	Donor AP31
7	LMDMQTFKA	LMDMQTFKA	LMDMQTFKA	LMDMQTFKA
8	KVSIPTKAL			KVSIPTKAL
9	SIPTKALEL			SIPTKALEL
11	TVSQKDVCL			
12	SVPNKALEL			
21	YLLHENCML	YLLHENCML	YLLHENCML	
24	QLQSKNMWL	QLQSKNMWL		QLQSKNMWL
28	SLSKILDTV	SLSKILDTV		SLSKILDTV
29	ILIDSGADI	ILIDSGADI	ILIDSGADI	ILIDSGADI
30	KVMEINREV			
32	AVYSEILSV			
34	ILSVVAKLL	ILSVVAKLL	ILSVVAKLL	ILSVVAKLL
37	SLTPLLLSI	SLTPLLLSI		SLTPLLLSI
38	SQYSGQLKV	SQYSGQLKV	SQYSGQLKV	SQYSGQLKV
40	QIMEYIRKL	QIMEYIRKL		QIMEYIRKL
49	SVPNKAFEL			
51	NLNYAGDAL	NLNYAGDAL		
54		GVTAEHYAV		
57		KSQEPAFHI		
59	MLKLEIATL	MLKLEIATL		MLKLEIATL
61		MLKKEIAML		
63	ALRIQDIEL			
67		VLKKKLSEA		
70	ELCSVRLTL	ELCSVRLTL	ELCSVRLTL	ELCSVRLTL
72	SLKINLNYA	SLKINLNYA		SLKINLNYA
74	ATCGMKVSI		ATCGMKVSI	
77	AELQMTLKL		AELQMTLKL	AELQMTLKL
78		VFAADICGV		
81	ILKEKNAEL	ILKEKNAEL		
84	NLVDVYGNM		NLVDVYGNM	
85	KCTALMLAV			

C. Immunological Reagents

Polyclonal antisera were generated against the following series of 22- to 23- mer peptides of BFA5:

BFA5(1-23) KLH-MTKRKKTINLNIQDAQKRTALHW (CLP-2977; SEQ ID NO: 99)
BFA5(312-334) KLH-TSEKFTWPAKGRPRKIAWEKKED (CLP-2978; SEQ ID NO: 100)
BFA5(612-634) KLH-DEILPSESKQKDYEENSWDTESL (CLP-2979; SEQ ID NO: 101)
BFA5(972-994) KLH-RLTLNQEEEKRRNADILNEKIRE (CLP-2980; SEQ ID NO: 102)
BFA5(1117-1139)KLH-AENTMLTSKLKEKQDKEILEAEI (CLP-2981; SEQ ID NO: 103)
BFA5(1319-1341)KLH-NYNNHLKNRIYQYEKEKAETENS (CLP-2982; SEQ ID NO: 104)

Prebleed samples from rabbits were processed and stored at –20°C. Rabbits were immunized as follows: 1) the peptides were administered as an emulsion with Freund's Complete Adjuvant (FCA); and, 2) two weeks later, the peptides were coupled with Keyhole-Limpet Hemocyanin (KLH)-coupled and administered as an emulsion with Freund's Incomplete Adjuvant FIA. The following results were observed:

TABLE V

Peptide/protein	IgG titer x 10^5 (After first Immunization Rb1/Rb2)	IgG titer x 10^5 (After second Immunization Rb1/Rb2)
CLP 2977	25/6	256/64
CLP 2978	25/25	64/256
CLP 2979	12/25	256/512
CLP 2980	25/12	1024/128
CLP 2981	8/4	256/64
CLP 2982	2/2	64/32

Prebleed sample results exhibited IgG titers <100 for all samples.

To assess the quality of the polyclonal antisera, western blots were performed using sera against BFA5. Sera were separately screened against cell extracts obtained from the BT474, MDMB453, MCF-7, Calu-6, and CosA2 cells. The approximate expected MW_r of BFA5 protein is 153 kDa. A 220kD band was observed in the BT474 extract with CLP2980 antibody but not in the MDMB453 cell extracts however a ~130kD band was present in the MDMB453 extract. Both bands were found to be consistent with the polyclonal antisera tested in this analysis. Neither of these bands is present in the negative control. Thus, it can be concluded that the polyclonal antisera are specific for BFA5.